

STUDY OF ANTIDIABETIC EFFECT OF POLYHERBAL EXTRACT IN ALLOXAN INDUCED DIABETIC RATS

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Article Info: Received 16 May 2020; Accepted 10 July 2020

DOI: <https://doi.org/10.32553/ijmbs.v4i7.1321>

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Conflict of interest: No conflict of interest.

Abstract

Diabetes mellitus is a metabolic disorder in which there is defect in insulin secretion high blood sugar levels over a prolonged period. The polyherbal extract under study contain extract of *Eugenia jambolana* (seed), *Citrus colocynthis* (fruit), *Terminalia chebula* (Fruit), *Curcuma amada* (Rhizome), *Syzygium cumini* (seed) in combination shows decrease in levels enhance gluconeogenesis from protein and Amino acid with accumulation of glycogen in liver. Crude drugs like *Eugenia jambolana*, *Mamordica charantia*, *Terminalia Chebula*, and *Black cumini* increase secretion of insulin from B-cells of islets of pancreas. The proposed study is to investigate the effectiveness that of combination of these herbs as a polyherbal extract formulation in treatment of type II diabetic model. In the present study, experimental diabetes was induced Alloxan dose 150 mg/kg (Group II), Diabetic standard diabetic rats were treated with oral dose of (metformin) 10mg/kg (Group III), group iv, v, vi) Diabetic rats were treated with an polyherbal extract 50mg/kg, 100mg/kg, 150mg /kg respectively. Drugs Metformin and polyherbal extract were given once in daily. The dietary regimen for 21 days, during which blood glucose level and lipid profile measured at 0,7,14,21 day of study using blood from rats retroorbital plexus. A high dose (200 mg/kg) of polyherbal extracts treatment group produce significant decrease in plasma glucose level.

Keywords: Diabetes, *Eugenia jambolana*, *Citrus colocynthis*, *Mamordica charantia*, *Gymnema sylvestre*

Introduction

Diabetes mellitus is one of the major disorders which are affecting the world's major population including the most vulnerable group like geriatrics and pediatric's population. Diabetes mellitus is a serious endocrine syndrome and complex chronic condition that is a major source of ill health worldwide. It was first reported in Egyptian manuscript about 3000 years ago [1]. In 1936, the distinction between type 1 and type 2 DM was clearly made [2]. Type 2 DM was first described as a component of metabolic syndrome in 1988 [3]. Type 2 DM (formerly known as non-insulin dependent DM) is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency [4]. Type 2 DM results from interaction between genetic, environmental and behavioral risk factors [5, 6]. People living with type 2 DM are more vulnerable to various forms of both short- and long-term complications, which often lead to their premature death. This tendency of increased morbidity and mortality is seen in patients with type 2 DM because of the commonness of this type of DM, its insidious onset and late recognition, especially in resource-poor developing countries like Africa [2,7]. This metabolic disorder is characterized by hyperglycaemia and disturbances of carbohydrate, protein and fat

metabolisms, secondary to an absolute or relative lack of the hormone insulin [1]. Diabetes mellitus is considered to be a serious in many countries it is traditional to use medicinal plants to control diabetes [2]. Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves. The most common is type 2 diabetes, usually in adults, which occurs when the body becomes resistant to insulin or doesn't make enough insulin. In the past three decades the prevalence of type 2 diabetes has risen dramatically in countries of all income levels. Type 1 diabetes, once known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin by itself. For people living with diabetes, access to affordable treatment, including insulin, is critical to their survival. There is a globally agreed target to halt the rise in diabetes and obesity by 2025.

About 422 million people worldwide have diabetes, the majority living in low- and middle-income countries, and 1.6 million deaths are directly attributed to diabetes each year. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades.

The synthetic hypoglycaemic agent can produce serious side effect and are not suitable in pregnancy whereas drug derived from plants are frequently consider to be less toxic with fewer side effects & affordable in price³ therefore the search for effective and safer hypoglycaemic agent has become area of research. This study has been undertaken to study the action of above mentioned plants polyherbal formulation on blood glucose level in alloxan induced diabetic rats.

The Polyherbal extract under investigation contain the extract of *Eugenia jambolana*(seed) ,*Mamordica charantia*(fruit) ,*Citrullus colocynthis* (fruit) , *Terminalia chebula* (fruit) ,*Curcuma amada* (Rhizome) ,*Syzgiumcumini*(seed) .in combination shows decrease in levels enhance gluconeogenesis from protein and amino acid with accumulation of glycogen in liver. Crude drugs like *Eugenia jambolana*, *Mamordica charantia* ,*Terminalia chebula* and *Black cumini* increase secretion of insulin from B- cells of islets of pancreas .Hence ,the proposed study is to investigate the effectiveness that of combination of these herbs as a Polyherbal extract formulation in treatment of Type II diabetic model .

Materials and methods

Animals

Male swiss albino rats (150-200g) were housed in the animal house of the institution under standardized condition (12 h light and 12 h dark cycle) and are provided free access to direct and purified drinking water *ad libitum*. All experimental procedures were carried out under strict compliance with Institutional Animal Ethical committee for the purpose of control and supervision of experimental, Animals (CPCSEA), Ministry of environment of forest; Government of India; New Delhi. Every possible effort was made to reduce the suffering of animals in all experimental design.

Chemicals

Alloxan monohydrate(aldrich pharma) , Metformin, Dextrose (Emkay Labs, India), Tween 80 (S. D. Fine-chem limited, Mumbai),Anaesthetic Ether (Ozone International, Mumbai).OneTouch® Select™ Glucometer, LifeScan, Inc.. One Touch select plus test strip (LifeScan, Inc.Pvt. Ltd. Mumbai). All other chemicals and reagents used were of analytical grade.

Plant materials

All the crude drugs were procured from the local market shop Wagh brothers, Nagpur. It was dried under shed at room temperature and finely powdered with the help of grinder. Further they are screened for phytochemical properties.

Preparation of extract

Extraction of all the crude drugs *Eugenia jambolna*(seeds), *Momordica charantia*(fruit), *Terminalia chebula*(seeds), *curcuma amada*(Rhizome), *Gymnema sylvestre*(leaves), *Citrullus colocynthis*(seeds) and *Black cumini*(seed) were prepared by the Maceration method:

Weighed 50 g of powdered drug were kept in a 500ml beaker to which 250 ml of distilled water was added and shaken for 6 h and kept for 7 days. The mixture was filtered through filter paper and filtrate was evaporated using evaporator for 40-45 and the residue was collected.

Composition of polyherbal extract

The polyherbal extract contains extract derived from: *Mamordica charantia* 250mg,*Eugenia jambolna* 250 mg , *Terminalia chebula* 100 mg, *citrullus colocynthis* 100 mg , *Black cumini* 100 mg , *Gymnema sylvestere* 100 mg ,*Curcuma amada*100mg , were weighed and made by using tween 80 as suspending agent volume was made by using saline and used further for animal study.

Table 1: Composition of Polyherbal extract

Sr. No.	Botanical name	Part used	Concentration
1	<i>Mamordicacharantia</i>	Fruit	250 mg
2	<i>Eugenia Jambolana</i>	Seed	250 mg
3	<i>Gymnemasylvestre</i>	Leaves	100mg
4	<i>Citrulluscolocynthis</i>	Seed	100 mg
5	<i>Terminaliachebula</i>	Seed	100 mg
6	<i>Curcuma amada</i>	Rhizome	100 mg
7	<i>Black cumini</i>	Seed	100 mg

EXPERIMENTAL INDUCTION OF DIABETES IN RATS:

The rats were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body wt intraperitoneally [13]. After 72 h, rats with moderate diabetes having glycosuria and hyperglycemia indicated by tests i.e. with blood glucose of 300-400 mg/dl were use for the experiment.

PRELIMINARY PHYTOCHEMICAL SCREENING:

Preliminary screening tests of extract were carried out for various plant constituents. The crude extract were tested for the presence or absence of secondary metabolites such as alkaloids, steroidal, phenolic compounds, flavanoids, saponins, Tannins and Anthraquinone using standard procedure [18].

Test for alkaloids

1.*Preliminary test:* A 100 ml of an extract was dissolved in dilute hydrochloric acid. Solution was clarified by filtration. Filtrate was tested with Drangendroffs and masters reagents. The treated solutions were observed for any precipitation.

2.*Confirmatory test:* Five gram of extract was treated with 40% calcium hydroxide solution until the extract was

distinctly alkaline to litmus paper, and then extracted twice with 10 ml portions of chloroform. Chloroform extracts were combined and concentrated in vacuum to about 5ml.

Test for steroidal compound

1. *Salkowskis test* – 0.5g extracts were dissolved in 2 ml chloroform in test tube. Concentrated sulfuric acid was carefully added on wall of test tube to form a lower layer. A reddish brown color at the interface indicated the presence of steroids ring (i.e., aglycon portion of the cardiac glycoside)

2. *Liebermann's test* – 0.5g of extract were dissolved in 2 ml of acetic anhydride and cooled well in an ice bath. Concentrate sulfuric acid was then carefully added. A color change from purple to green indicated the presence of steroid nucleus, i.e., aglycone portion of cardiac glycoside.

Test for phenolic compounds

1. The extract were dissolved in water Few crystal of ferric sulphate were added to the mixture. Formation of dark violet color indicated the presence of phenolic compounds.

Flavonoids

1. *Lead acetate test*. To a solution 0.5g extract in water about 1 ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.

Test for Saponin

1. *Froth test* – 0.5g extracts were dissolved in 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 sec. The test tube was allowed to stand in vertical position and observed over 30min period of time.

Test for tannins

1. *Ferric chloride test* – a portion of extracts were dissolved in water. The solution was clarified by filtration, 10% ferric chloride solution was added to the clear filtrate. This was observed for for a change in color to bluish black.

Test for Anthraquinone

1. Test for free Anthraquinone (Borntragger test). The Aqueous extract of plant material was shaken vigorously with 10 ml of benzene, filtered, and 5ml of 10% ammonia solution added to the filtrate. Shake the mixture and presence of a pink, red, or violet color in ammonia (lower) phase indicated the presence of free Anthraquinones.

2. Test for anthraquinone glycoside (Modified Borntragger test)

5 g of plant extract were boiled with 10 ml 5% sulfuric acid for 1hr and filtered while hot. The filtrate was shaken with 5 ml benzene layer separated and half its own volume of

10% ammonia solution added. The formation of pink, red, violet color in ammonia phase (lower layer) indicated the presence of Anthraquinone derivative in the extract.

BIOCHEMICAL ESTIMATION:

Blood samples was collected from retro orbital plexus and collected in micro centrifuge tubes which were centrifuged at 5000 rpm for 10 min using cooling centrifuge. The supernatant was use for measurements of glucose and lipid profile (Fleming, q1972)

Glucose oxidase peroxide method

Blood glucose was estimated by commercially available glucose kit base on the Glucose oxidase method. Label the clean dry test tube as Blank (B), Standard (S), and Test (T):

Table 2: Quantity to be used for estimation

Samples	B(ml)	S (ml)	T (ml)
Glucose reagent	1.0	1.0	1.0
Glucose Standard	-	0.01	-
Sample	-	-	0.01

Stirring well and incubate at 37 °c for 10min or at R. T (25 °c) for 30min. Measure at 510 NM of the standard (s) and Test sample (T) against the blank (B) within 60min. Calculate the result by using formula.

$$\text{Serum Plasma glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

Cholesterol estimation method

Pipette into Clean dry test tube lanes as Blank (B), Standard (S), and Test (T):

Table 3: Quantity used for the estimation

Reagent	B(ml)	S(ml)	T(ml)
Cholesterol reagent	1.0	1.0	1.0
Cholesterol standard	-	0.01	-
Sample	-	-	0.01

Mix well and incubate at 37 °c for 10 min or at R. T (25 °c) for 30min. Measure Absorbance at 510 nm of the standard (s) and Test sample (T) against the blank (B) within 60min. Calculate the result by following formula.

$$\text{Serum Plasma glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

Triglycerides Estimation Method:

Pipette into Clean dry test tubes label as blank (B), standard (s) and Test (T) :

Table 4: Quantity to be taken for analysis

Reagent	B(ml)	S(ml)	T(ml)
Triglycerides reagent	1.0	1.0	1.0
Triglycerides standard	-	0.01	-
Sample	-	-	0.01

Mix well and incubate at 37^oc for 10mini at R .T (25^oc) for 30 min .Measure Absorbance at 510 NM of standard (s) and Test sample (T) against the blank (B) within 60 min. Calculate the result by following formula.

$$\text{Serum Plasma glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

Experimental design

In the experiment, diabetes was induced in rats 2 weeks before starting the experiment. The rats were divided into four groups after the induction of alloxan diabetes. In the experiment six rats were used in each group.

Group I: Normal control rat treated with vehicle daily.

Group II: Diabetic control given Alloxan dose 150 mg/kg and treated with vehicle.

Group III: Diabetic standard diabetic Rats were treated with oral dose of (Metformin) 100 mg/kg.

Group IV: Diabetic rats were treated with of Polyherbal extract 50 mg /kg. /Po

Group V: Diabetic rats were treated with of Polyherbal extract 100 mg/kg /Po

Group VI: Diabetic rats were treated with of Polyherbal extract 200 mg /kg/Po .

The drugs metformin and polyherbal extract were given once a daily.

The dietary regimen lasted for 21 days, during which blood glucose level and lipid profile measured at 0, 7th, 14th, and 21th day of the study using blood from rats retro orbital plexus . At the end of experiments rats were fasted overnight, and killed.

Results

Phytochemical screening of crude drugs:

Table 5: Percentage yieldof crude drugs

Drugs	% yield
<i>Mamordica charantia</i>	0.5 % w/w
<i>Eugenia jambolana</i>	2 %w/w
<i>Gymnema sylvestre</i>	5.5 % w/w
<i>Citrullus colocynthis</i>	3. 45% w/w
<i>Curcuma amada</i>	0.34% w/w
<i>Black cumini</i>	4.5 % w/w
<i>Terminalia chebula</i>	5% w/w

Table 6: Qualitative test of crude drugs

Test	Reagents	M.C.	E.J.	T.C.	C.A.	C.C	G.S.	B.C.
Test for alkaloids	Dragendroff's reagent	+	+	-	-	+	+	+
Test for Steroids	Acetic acid +conc .sulphuric acid	+	-	-	-	-	-	-
Test for tannins	FeCl ₃ solutions	+	+	+	+	+	+	+
Test for phenolic compound	5 % FeSO ₄	-	-	-	-	-	-	-
Test for anthraquinone Glycoside	Conc. H ₂ SO ₄ solution and CHCl ₃	-	-	-	-	-	-	-
Test for carbohydrate	Molish reagent	+	+	+	+	+	+	+

Note: Ethanolic extract of *Mamordica charantia* (M.C), Ethanolic extract of *Eugenia jambolna* (E.J), aqueous extract of *Terminalia chebula* (T.C.), aqu. extract of *Gymnemastylvestre* (G.S.),aqu. & methanolic extract of *Curcuma amada* (C.A.),aqu. extract of *Citrulluscolocynthis* (C.C.).

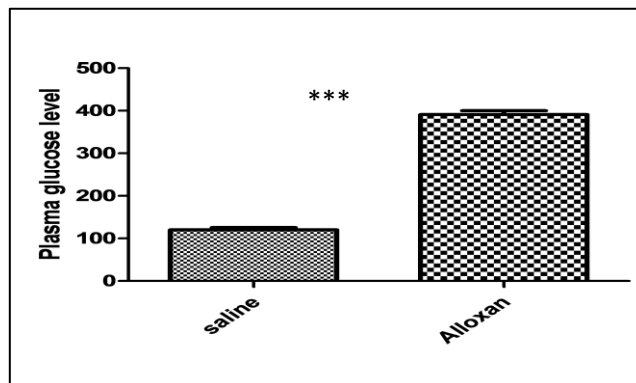
(+) Positive test ;(-) Negative test

Standardisation of model

Table7: Effect of alloxan on plasma Glucose level

Group	Day 9	Day 14	Day 21
Control	120±0.23	129.83±0.28	110.16±0.28
Diabetic control	399.5±0.39	401.16±0.64	372.83±11.6

After 72 hr. on alloxan induce Diabetic model values are represented as mean \pm S.E.M (n = 6) * * * P < 0.001 as compared to saline group Data analyzed one way anova followed by Bonferroni's Multiple Comparison Test "

**Figure 1:** Effect of alloxan on Plasma Glucose level

Plasma Blood Glucose level:

Table 8: Plasma glucose level (mg/ml)

Group	Day 9	Day 14	Day 21
Control	120±0.23	129.83±0.28	110.16±0.28
Diabetic control	399.5±0.39	401.16±0.64	372.83±11.6
Slandered	370.16±0.28	201±0.47	140.33±0.30
P.E. 50 mg/kg	332.83±0.43	290±0.33	200.33±0.30
P.E. 100 mg/kg	306.67±0.38	300±0.53	190.83±0.28
P.E. 200mg /kg	340.16±0.28	210.16±0.36	154.5 ± 0.39

After 21 days in alloxan induce Diabetic model values are represented as mean \pm S.E.M (n = 6) * * * P< 0.001 as compared to saline group Data analyzed one way anova followed by Bonferroni's Multiple Comparison Test "

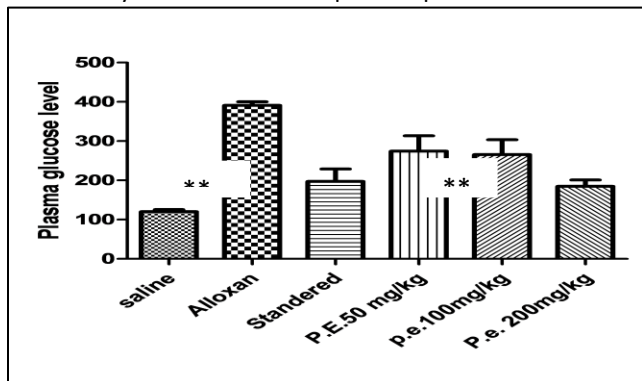


Figure 2: Effect of Polyherbal extract on plasma Glucose level

Table 9: Plasma SGOT level

Group	Day 9	Day 14	Day21
Control	23.83±0.28	23±0.33	25.16±0.28
Diabetic control	50.16±0.28	59.83±0.43	44.66±0.56
Standard	50.16±1.54	48.33±2.8	.33.33±0.30
P.E. 50 mg/kg	74.83±0.28	43.33±0.45	43.33±0.38
P.E. 100 mg/kg	75.16±0.280542	50.83±0.28	45.83±0.28
P.E. 200 mg/kg	70.16±0.35	65±0.33	34.5±0.39

After 21 days in alloxan induce Diabetic model values are represented as mean \pm S.E.M (n = 6) * * * P< 0.001 as compared to saline group Data analyzed one way anova followed by Bonferroni's Multiple Comparison Test "

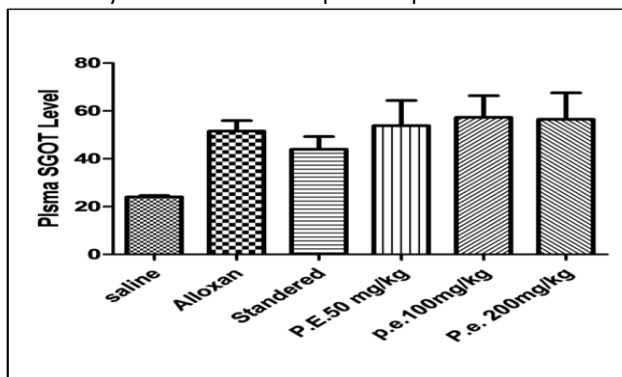


Figure 3: Effect of Polyherbal extract on Plasma SGOT level

Table 10: Plasma SGPT level

Group	Day 9	Day 14	Day 21
Control	30.83 ±0.28	22.66±0.30	25.5±0.20
Diabetic control	64.83 ±0.28	61.66±0.60	64.83±1.03
Standard	64.66±0.38	35.16±0.28	36.66±0.65
P.E. 50 mg/kg	54.66±0.30	45.33±0.19	50.83±0.28
P.E. 100 mg/kg	64.66±0.38	48.16±0.43	43.16 ±0.28
P.E. 200mg /kg	64.83±0.28	52.5±0.69	35.33±0.30

After 21 days in alloxan induced Diabetic model values are represented as mean \pm S.E.M (n = 6) * * * P< 0.001 as compared to saline group Data analyzed one way anova followed by Bonferroni's Multiple Comparison Test "

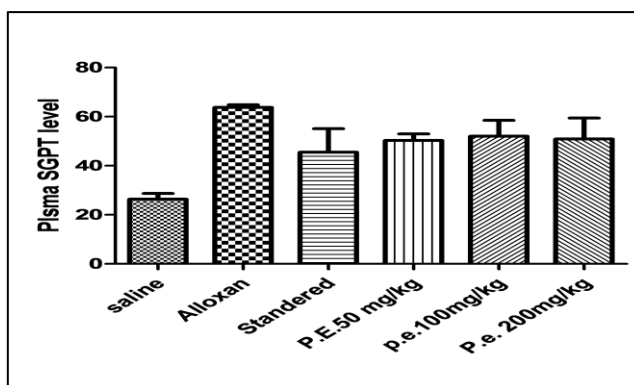


Figure 4: Effect of Polyherbal extract on plasma SGPT level

Discussion

Diabetes mellitus is one of most common chronic disease that associated with various complications. Alloxan, a beta cytotoxin, induces 'chemical diabetes' (Alloxandiabetes in a wide variety of animals by damaging the insulin secreting B-cells of pancreas, and reduce the release of insulin that leads to hyperglycemia in alloxan induced diabetes rats.

In the present study, it was observed that polyherbal extract decreases the blood glucose level in alloxan diabetic rats. The mechanism of action of polyherbal extract correlates with oral hyperglycemic drugs that increase secretion of insulin from pancreas. In this context, number of other plants has also been reported to have antihyperglycemic and insulin stimulatory effects. Like plant extract, Metformin also produced significant reduction in alloxan diabetic rats. Since, alloxan is known to destroy pancreatic B-cells destruction.

The result shows increased Plasma glucose level; SGOT and SGPT level of diabetic control group. A high dose (200mg/kg) of treatment group produce significant decrease in Plasma glucose level but not shows decrease in SGOT and SGPT level significantly. Administration of polyherbal extract and Metformin reduced the blood glucose level of diabetic rats. This could correlates with previous study which reported that *Eugenia jambolana*, *Mamordicacharantia*, *Citrulluscolocynthis*, *Curcuma amada*, *Terminaliachebula*, *Black cumini*, *Gymnemastylvestreshows* antidiabetic activity may be due to the effect of active constituents of different plants, viz, momordin, charantin (Alkaloids) from *Mamordicacharantia*, gallic acid, ellagic acid from *Eugenia jambolana*, chebulin from *Terminaliachebula*, curcuminoids and phenolic acids from *curcuma amada*

,gymnemic acid from *GymnemaStylvestre* ,and linoleicacid from *Black cumini* .

On the basis of above result, it could be concluded that Polyherbal extract, a combination of seven herbal plants exert significant antihyperglycemic activity.

Conclusion

The present study demonstrated that this polyherbal extract exhibits promising hypoglycemic activity and help to maintain good glycemic and metabolic control. The polyherbal extract elicits hypoglycemic effects in both normal and experimentally induced hyperglycemic rats .The polyherbal extract exhibited significant antihyperglycemic activity in alloxan induce diabetic rats.

Declarations

Ethical approval and consent to participate (human data): Not applicable

Ethical approval and consent to participate (animal studies): Applicable

Consent for publication: Not applicable

Availability of data and material: Yes

Competing interests: Not applicable

Funding: Not applicable

Authors' contributions:

SM designed the present study, PK performed the experiments and VB had drafted the manuscript. All the authors read and approved the final manuscript.

Acknowledgements:

All the authors liked to show their gratitude to the Priyadarshini J.L. College of Pharmacy, Nagpur for providing all the facilities to performed research work.

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